WEST

End of Result Set

Generate Collection

L3: Entry 1 of 1 File: USPT Sep 23, 1997

US-PAT-NO: 5670340
DOCUMENT-IDENTIFIER: US 5670340 A

TITLE: Process for producing peptides in E. coli

DATE-ISSUED: September 23, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yabuta; Masayuki	Tatebayashi	N/A	N/A	JPX
Suzuki; Yuji	Ashikaga	N/A	N/A	JPX
Ohsuye; Kazuhiro	Ohra-gun	N/A	N/A	JPX
Oshima; Takehiro	Ashikaga	N/A	N/A	JPX
Onai; Seiko	Isesaki	N/A	N/A	JPX
Magota; Koji	Takatsuki	N/A	N/A	JPX
Tanaka; Shoji	Ashiya	N/A	N/A	JPX

US-CL-CURRENT: 435/69.4; 435/68.1, 435/69.1, 435/69.7

CLAIMS:

We claim:
1. A process for the production of a (target peptide) comprising:
A) culturing E. coli host cells transformed with a plasmid capable of expressing a gene coding for a fusion protein represented by the following formula:
A--L--B
wherein, B is a target peptide selected from the group consisting of calcitonin precursor, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide, A is a protective peptide comprising a fragment of 90-210 amino acids from the N-terminal of the E. coli .beta.-galactosidase polypeptide to protect the peptide to which it is fused, and L is a linker peptide positioned between the C-terminal of said protective peptide and the N-terminal of said target peptide and is selected such that when said fusion protein is treated with an enzyme or chemical substance, said target peptide is cleaved from said linker peptide, and wherein the isoelectric point of the entire fusion protein A--L--B is adjusted to a range between 4.9 and 6.9 by including a linker peptide having basic amino acid residues;
B) obtaining an insoluble fraction comprising inclusion bodies by homogenization of said transformed E. coli host cells;
C) solubilizing said fusion protein comprised in said inclusion bodies by treatment of said insoluble fraction with a solubilizing agent; and,
D) cleaving the peptide bond between the C-terminal of the linker amino acid residue and the N-terminal of the target peptide contained in said solubilized fusion protein to release said target peptide from said fusion peptide.
2. A process according to claim 1, wherein the calcitonin is human calcitonin.
3. A process according to claim 1, wherein the C-type natriuretie peptide is C-type natriuretie peptide-22.
4. The method of claim 1 wherein the fragment of the .beta.-galactosidase polypeptide comprises the 97 amino-terminal amino acids of .beta.-galactosidase.
5. The method of claim 4 wherein the fragment comprising the 97 amino-terminal amino acids of .beta.-galactosidase has been mutated to change a cysteine to a serine residue and further to change four glutamic acid residues to aspartic acid residues.

residues.

WEST

End of Result Set

Generate Collection

L1: Entry 1 of 1 File: USPT Apr 13, 1993

US-PAT-NO: 5202239
DOCUMENT-IDENTIFIER: US 5202239 A

TITLE: Expression of recombinant polypeptides with improved purification

DATE-ISSUED: April 13, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tarnowski; S. Joseph	Sunnyvale	CA	N/A	N/A
Hilliker; Sandra	Riverside	CA	N/A	N/A
Willett; W. Scott	San Francisco	CA	N/A	N/A

US-CL-CURRENT: 435/69.7; 435/252.3, 435/320.1, 530/350, 530/412

CLAIMS:

What is claimed is:
1. A method for producing a purified peptide, said method comprising:
(a) expressing a gene encoding a fusion protein within a host cell, wherein said fusion protein comprises
(i) a carrier protein of about 10 to about 50 kDa which does not contain Glu residues or Asp-Gly sequences as a Staph V8 cleavage site, wherein said carrier protein consists essentially of the sequence: ##STR2## wherein X.sub.1-9 are each independently selected from the group consisting of Ala, Asp, Cys, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr; and X.sub.10 and X.sub.11 are each independently selected from the group consisting of Ala, Arg, Asp, Cys, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Ser, Thr, Val, Trp, and Tyr, with the proviso that X.sub.11 is not Asp, and if X.sub.10 is Asp then X.sub.11 is not Gly;
(ii) a Staph V8 cleavage site positioned at the C-terminal of said carrier protein; and
(iii) said peptide, not containing a Staph V8 cleavage site, fused to said cleavage site;
wherein said fusion protein exhibits a pI of about 8.0 or greater;
(b) separating said fusion protein from proteins endogenous to said host cell by a process, at least one step of which consists essentially of cation exchange chromatography under conditions wherein said fusion protein is adsorbed to a cation exchange column while proteins endogenous to said host cells are not adsorbed to said column;
(c) eluting said fusion protein;
(d) cleaving said fusion protein at said cleavage site to liberate said carrier protein from said peptide; and
(e) recovering said peptide free from said carrier protein.
2. The method of claim 1, wherein X.sub.1-9 are each Gln.
3. The method of claim 2, wherein said fusion protein further comprises an N-terminal leader of about 6 to about 20 amino acids.
4. The method of claim 3, wherein said leader comprises about 3 to about 9 Thr residues.
5. The method of claim 4, wherein said leader consists essentially of the sequence: ##STR3##
6. The method of claim 5, wherein said peptide is selected from the group consisting of ANP, brain natriuretic peptide, somatostatin, glucagon-like peptide, calcitonin, lung surfactant, insulin, growth hormone releasing factor, bradykinins, endorphins, and enkephalins.

bradykinins, endorphins, and enkephalins.
7. The method of claim 6, wherein said peptide is human ANP or an analog of ANP.
8. The method of claim 7, wherein said peptide consists essentially of the sequence: ##STR4##

WEST

End of Result Set

Generate Collection

L2: Entry 1 of 1 File: USPT Jun 21, 1994

US-PAT-NO: 5322930
DOCUMENT-IDENTIFIER: US 5322930 A

TITLE: Expression of recombinant polypeptides with improved purification

DATE-ISSUED: June 21, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tarnowski; S. Joseph	Sunnyvale	CA	N/A	N/A
Hilliker; Sandra	Riverside	CA	N/A	N/A
Willett; W. Scott	San Francisco	CA	N/A	N/A

US-CL-CURRENT: 530/350; 435/252.3, 435/320.1, 435/69.7, 530/369, 536/23.4

CLAIMS:

What is claimed is:
1. A fusion protein designed for improved purification, which fusion protein comprises, from N-terminal to C-terminal:
a carrier protein of about 10 to about 50 kDa which does not contain accessible Glu residues or Asp-Gly sequence as a cleavage site;
a Staph V8 cleavage site for Glu residues or Asp-Gly sequences positioned at the C-terminal of said carrier; and
a peptide not containing Glu residues or Asp-Gly sequences as a cleavage site, fused to said cleavage site, wherein said fusion protein exhibits a pI of about 8.0 or greater;
wherein said carrier protein consists essentially of the sequence: ##STR2##
wherein X.sub.1-9 are each independently selected from the group consisting of Ala, Asp, Cys, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr; and
X.sub.10 and X.sub.11 are each independently selected from the group consisting of Ala, Asp, Cys, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr, with the proviso that X.sub.11 is not Asp, and if X.sub.10 is Asp then X.sub.11 is not Gly.
2. The fusion protein of claim 1, wherein X.sub.1-9 are each Gln.
3. The fusion protein of claim 2, wherein said fusion protein further comprises an N-terminal leader of about 6 to about 20 amino acids.
4. The fusion protein of claim 3, wherein said leader comprises about 3 to about 9 Thr residues.
5. The fusion protein of claim 4, wherein said leader consists essentially of the sequence:
Met-Thr-Met-Ile-Thr-Asn-Leu-Thr-Thr-Thr-Thr-Thr-Thr-Gln-Phe-Arg-Met-.
6. The fusion protein of claim 5, wherein said peptide is selected from the group consisting of ANP, analogs of ANP, brain natriuretic peptide, somatostatin, glucagon-like peptide, calcitonin, lung surfactant, insulin, growth hormone releasing factor, bradykinins, endorphins, and enkephalins.
7. The fusion protein of claim 6, wherein said peptide is human ANP.
8. The fusion protein of claim 5, wherein said peptide consists essentially of the sequence:
Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Met-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu--Gl
y-Cys-Asn-Ser-Phe-Arg-Tyr.